PROTEIN DIFFERENCES ASSOCIATED WITH THE ABSENCE OF GRANULE CELLS IN THE CEREBELLA FROM THE MUTANT WEAVER MOUSE AND FROM X-IRRADIATED RAT

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1. Introduction

The development of synaptic connections in the nervous systems of vertebrates [1] and invertebrates [2,3] is subject to a stringent genetic determinism which governs, for instance, the differentiation and migration of neurons, the growth of their processes and the selective recognition between pre- and post-synaptic surfaces. Nevertheless, there exists some evidence that the final connectivity and the functional state of some particular synapses, in both central and peripheral nervous systems, might be modulated by their activity at critical stages of development (see ref. [4,5]).

In spite of its complex structure, the cerebellum constitutes a particularly suitable region of the brain to study these processes and, in particular, to distinguish between genetic and 'epigenetic' [5] phenomena. Its anatomy is rather well understood [6,8]; the electrophysiological activity of single neurons can be recorded [6,9]; most of its development occurs postnatally and becomes therefore easily accessible to the experiment; it contains only a few classes of cells but these cells are repeated a large number of times and therefore amenable to direct biochemical analysis.

In the mouse, a number of mutations have been identified and mapped which lead to profound alterations of cerebellum anatomy [10] and therefore of its physiology [11]. In addition, one can induce some phenocopies of these mutations, such as the lack of granule cells, by drug injection [12–15], infection with specific viruses [16] or X-irradiation [17,18].

In this paper, we describe experiments done with agranular cerebella from both the mutant *weaver* (wv) mouse [19–24] and X-irradiated rats. The electrophoretic patterns of the proteins solubilised from subcellular fractions of these cerebella are compared with those obtained with normal mouse and rat. Striking differences are noticed and, by comparison with the patterns obtained with a preparation of purified rat granule cells, assigned to the lack of granule cells.

2. Materials and methods

2.1. *Animals*

2.1.1. Mutant 'weaver' mouse

The stock of mice B6CBA/51 originating from the Jackson Laboratory (Bar Harbor, Maine, USA) was raised at the Pasteur Institute. Homozygous w/wv were obtained by intercrossing of heterozygous wv/+ mice and distinguished from their littermates on the basis of two criteria: a) abnormal behavior: ataxia, hypotonia and fine tremor which lack in wv/+ and +/+ animals [20], and b) marked reduction in cerebellar size: about 70% decrease in the area of the vermis in the mid-sagittal plane compared to 5–10% reduction in wv/+ [21]. The animals used as controls derived from littermates which did not show any abnormal behaviour nor reduction in cerebellar size and were intercrossed for five generations without giving the *weaver* phenotype in their progeny.

Animals at an age of 21 days were used.

2.1.1. X-irradiated rats

Rats from the Sherman strain received a total dose of 1200 rad given as follows: 200 rad at birth and on the 3 and 5 days, and 150 rad on the 7, 10, 12 and 14 days. Two rats of 21 days weighting respectively 20 g and 22 g were used in the experiments. Non-irradiated littermates were taken as controls. The agranular rats were a gift of Dr Nicole Delhaye-Bouchaud.

2.2. *Preparation of subcellular fractions from mouse and rat cerebella*

The animals were killed without anesthesia and their cerebellum dissected immediately and mixed with 10^{-3} M Na phosphate buffer pH 7.0 at 4°C, and homogenized in a motor-driven glass-teflon Potter homogenizer with 50 up and down strokes. The homogenate was then centrifuged at maximum speed for 20 min in a Beckman 152 microfuge. The supernatant was collected and the pellet resuspended with the aforementioned buffer and recentrifuged at maximum speed in the microfuge for 20 min giving the pellet P1. The first supernatant was then centrifuged at 30 000 rpm in a rotor type 65 for 74 min in a Beckman model L2-65 ultracentrifuge, giving a pellet P2 and a final supernatant S. P1, P2 and S were dissolved in a 10% solution of sodium dodecylsulphate containing 1% β-mercaptoethanol and brought to boiling for a few minutes.

2.3. *Preparation of granule cells from rat cerebellum*

Granule cells were purified from 12–13 days OFA rats by the method of Sellinger et al. [25] except that bovine serum albumin was replaced by 1% polyvinyl pyrolidone (PVP). The granule cell fraction collected from the final sucrose gradient was diluted 4-fold with 10^{-3} M Na phosphate buffer at pH 7.0 and centrifuged at 1000 *g* for 15 min. The pellet was then dissolved in a 10% SDS solution containing 1% mercaptoethanol brought to boiling for a few minutes.

2.4. *Slab gel electrophoresis*

Slab gel electrophoresis in 10% polyacrylamide-SDS was carried out using the procedure of Ames [26]. Each sample contained 15–25 μg protein. The electrophoresis was run at room temperature at a constant current of 15 mA.

Gels were fixed in isopropanol–acetic acid to remove SDS, stained in Coomasie Blue in methanol–acetic acid–water and destained in methanol–acetic acid–water. Molecular weight calibration was done with: rabbit phosphorylase *b* (94 000), bovine serum albumin (68 000), glutamate dehydrogenase (53 000); aldolase (40 000), chymotrypsinogen (25 000) and Kunitz trypsine inhibitor (20 100).

3. Results

Slab gel electrophoresis in SDS has been carried out with subcellular fractions: low (P1) and high (P2) speed pellets and supernatant S (see Materials and methods) prepared from the cerebella of homozygous 'weaver' wv/wv mouse, X-irradiated rat and of control animals of the same age (21 days).

The most salient differences between control and experimental animals appeared in the low speed particulate fractions P1 and only results concerning this fraction are reported here. Fig. 1 (top) shows the protein patterns of the P1 fractions from normal (n) and X-irradiated (x) rats. In the fraction from X-irradiated rat three bands (no. 1, 2 and 3) were consistently absent (or much less intense) while another one (no. 4), in some gels, appeared darker than in the control.

A preparation of granule cells purified from 12–13 days rats was run on the same gel. The protein pattern strikingly differed from that found with P1 from both normal and X-irradiated rats: the total number of bands was smaller and only a few stained intensely. These bands have the same mobility as those missing in the X-irradiated cerebellum: no. 1, 2 and 3. Mixing the P1 fraction from normal rats and granule cells resulted in a reinforcement of those three bands. Thus we associate protein bands 1, 2 and 3 with granule cells. The apparent mol. wt. of these bands was measured by comparison with globular proteins of known mol. wt. (fig. 2) and found to be: $1.0 \pm 0.2 \times 10^5$, $3.1 \pm 0.1 \times 10^4$ and $2.8 \pm 0.1 \times 10^4$ for the bands 1, 2 and 3 respectively.

Similar results were obtained with the agranular cerebellum from the wv/wv mouse as shown on fig. 1 (bottom). Minor differences were noticed between the protein patterns of the P1 fraction from control mouse and rat.

The band no. 3 appeared to have a slightly higher

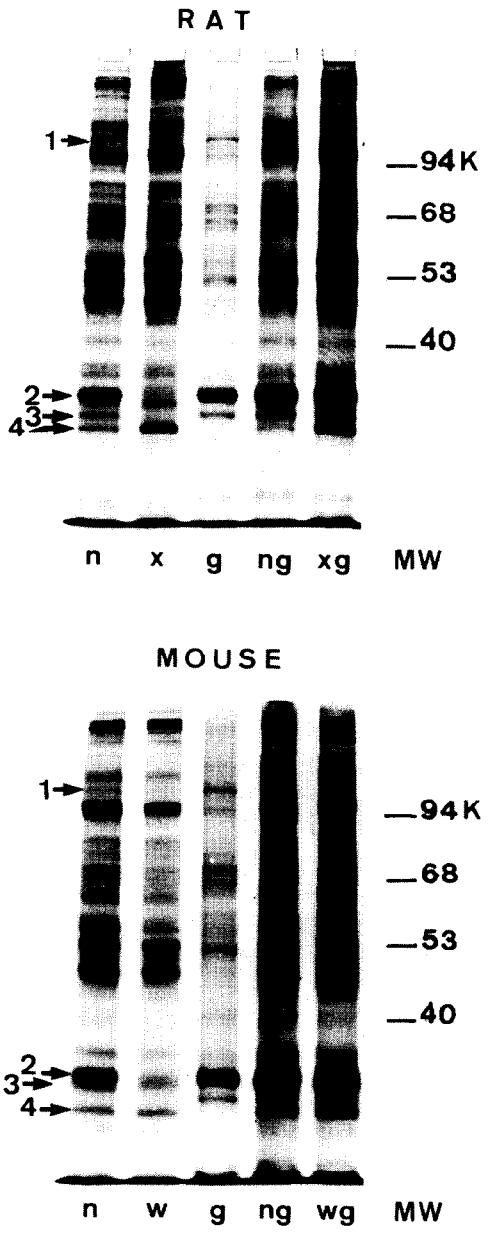

Fig. 1. Slab gel electrophoresis in the presence of SDS of the particulate fraction P1 prepared from X-irradiated rat (X) and mutant weaver mouse (w) cerebella. On the same gel were run P1 fraction from normal (n) mouse and rat cerebella and granule cells (g) purified from rat cerebellum. The granule cells preparation was mixed (50/50) with P1 extracts from normal mouse or rat (ng) and from X-irradiated rat (xg) or mutant weaver mouse (wg).

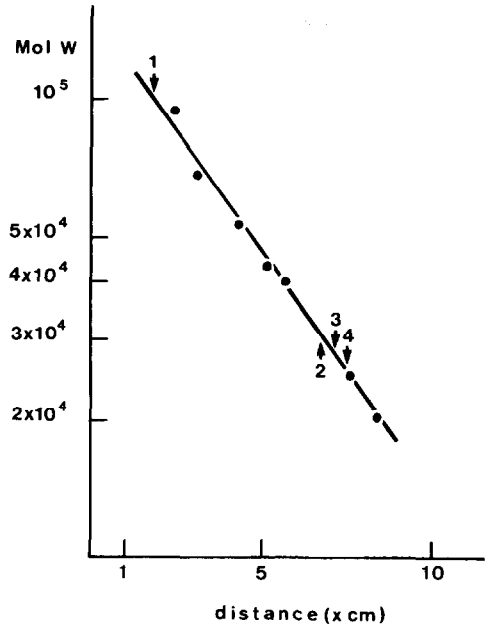

Fig. 2. Determination of apparent molecular weights of the proteins which appear modified in X-irradiated rat and make the bands 1, 2, 3 and 4. The dots correspond to globular proteins of known mol. wt. used as standards. In abscissa is plotted the distance migrated on the gel slab.

mol. wt. ($2.9–3.0 \times 10^4$ vs 2.8×10^4) in the mouse than in the rat. The bands no. 1 and 2 which were missing in the P1 fraction of X-irradiated rat cerebellum, were also missing in the P1 fraction prepared from the wv/wv mouse (w). They have the same mobility and, therefore the same apparent molecular weight as those found with rat purified granule cells. The absence of bands no. 1 and 2 appears therefore characteristic of the deficit in granule cells of both X-irradiated rat and mouse wv/wv cerebellum.

4. Discussion

We have described changes in the protein pattern of particulate fractions from mouse and rat cerebellum which coincide with the absence of one class of cerebellar neurons, the granule cells. At present nothing can be said about the nature of the protein species concerned except that they are major components in purified granule cells. By electron microscopy, the

surface of the granule cells frequently appears damaged. The preparation also contains a significant fraction of isolated granule cell nuclei. It is possible that the 3.1×10^4 mol. wt. protein is a nuclear protein. Immunological studies are in progress in this laboratory to test this point and to study the distribution of these proteins in the various regions of the cerebellum and of the brain.

Changes in protein pattern have also been reported in other nerve tissues as a consequence of mutation [27–30] or disease [31,32] but not yet related to alterations in the properties of a given class of neuron. Our approach of identifying protein markers for specific cell types should be useful for the study and manipulation of cell migration and contact formation in the brain.

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